EXHIBIT A140

Supplemental Selenium May Decrease Ovarian Cancer Risk in African-American Women[1–3]

Paul D Terry,[4]* Bo Qin,[5] Fabian Camacho,[6] Patricia G Moorman,[7] Anthony J Alberg,[8] Jill S Barnholtz-Sloan,[9] Melissa Bondy,[10] Michele L Cote,[11] Ellen Funkhouser,[12] Kristin A Guertin,[6] Edward S Peters,[13] Ann G Schwartz,[11] Joellen M Schildkraut,[6] and Elisa V Bandera[5]

[4]Department of Medicine, Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN; [5]Department of Population Science, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; [6]Department of Public Health Sciences, University of Virginia, Charlottesville, VA; [7]Department of Community and Family Medicine, Duke Cancer Institute, Durham, NC; [8]Hollings Cancer Center and Department of Public Health Sciences, Medical University of South Carolina, Charleston, SC; [9]Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH; [10]Cancer Prevention and Population Sciences Program, Baylor College of Medicine, Houston, TX; [11]Department of Oncology and the Karmanos Cancer Institute, Population Studies and Disparities Research Program, Wayne State University School of Medicine, Detroit, MI; [12]Division of Preventive Medicine, University of Alabama at Birmingham, Birmingham, AL; and [13]Epidemiology Program, Louisiana State University Health Sciences Center School of Public Health, New Orleans, LA

Abstract

Background: To our knowledge, no previous study has evaluated the associations of antioxidant intake with the risk of ovarian cancer in African-American women, who are known to have high mortality from the disease.

Objective: We sought to evaluate these associations among 406 ovarian cancer cases and 632 age- and site-matched controls of African-American descent recruited from AACES (African American Cancer Epidemiology Study), a population-based, case-control study in 11 geographical areas within the United States.

Methods: Multivariable logistic regression models were used to estimate ORs and 95% CIs adjusted for a wide range of potentially confounding factors, including age, region, education, parity, oral contraceptive use, menopause, tubal ligation, family history, body mass index (BMI), smoking status, total energy, and physical activity.

Results: Women with the highest intakes of supplemental selenium (>20 μg/d) had an ∼30% lower risk of ovarian cancer than those with no supplemental intake (OR: 0.67; 95% CI: 0.46, 0.97; P-trend = 0.035). This inverse association was stronger in current smokers (OR: 0.13; 95% CI: 0.04, 0.46; P-trend = 0.001). There was no association with dietary selenium. The associations with carotenoid intakes were weak and nonsignificant (P = 0.07–0.60). We observed no association with dietary or supplemental intake of vitamin C or vitamin E. There were no appreciable differences in results between serous and nonserous tumors.

Conclusions: These findings provide the first insights, to our knowledge, into the potential association between antioxidants and ovarian cancer in African-American women, indicating potential inverse associations with supplemental selenium. *J Nutr* 2017;147:621–7.

Keywords: antioxidants, diet, ovarian cancer, African American, women

Introduction

Ovarian cancer is a leading cause of gynecologic cancer death (1). Given the relatively poor survival of women diagnosed with the disease and the lack of effective screening tools, identifying modifiable risk factors is essential to reducing ovarian cancer mortality and morbidity. The etiology of ovarian cancer is multifactorial, encompassing reproductive, hormonal, genetic, environmental, and lifestyle factors. Among dietary risk factors, antioxidant compounds have been of interest. A few studies have suggested that a high consumption of fruit and vegetables may decrease ovarian cancer risk, as may the use of antioxidant supplements (2). An imbalance between free radicals and antioxidants (oxidative stress) can lead to the formation of genotoxic lipid peroxidation byproducts and contribute to progression of ovarian carcinogenesis (3). However, the number of dietary studies of ovarian cancer is relatively small, and studies have been conducted almost exclusively in Caucasian women. Hence, the association between antioxidant intake and ovarian cancer risk in African-American women is virtually unexplored. This may be an important gap in knowledge given the results of studies that suggest the possibility of higher levels of oxidative stress in African Americans (4, 5), lower intakes of antioxidants (6, 7), different lifestyle correlates of antioxidant levels (8), and stronger associations between antioxidant intake and cancer risk in African Americans (6, 9). To address this lack of evidence and to allow comparisons with other populations,

621

we evaluated associations of commonly studied antioxidants with risk of ovarian cancer in a large, population-based, multi-center case-control study of women enrolled in AACES (African American Cancer Epidemiology Study).

Methods

Design and participants. This study was conducted among women of African-American descent recruited into AACES, an ongoing, population-based, case-control study of ovarian cancer in 11 sites in the United States (Alabama, Georgia, Illinois, Louisiana, Michigan, North Carolina, New Jersey, Ohio, South Carolina, Tennessee, and Texas) (10). Cases were identified by rapid case ascertainment utilizing state cancer registries; Surveillance, Epidemiology, and End Results Program registries; or hospitals' gynecologic oncology departments. Eligible cases include all self-identified African-American women between 20 and 79 y of age with newly diagnosed, histologically confirmed invasive epithelial ovarian cancer. Controls who self-identified as African American were selected by using random-digit dialing and were frequency-matched to cases by 5-y age groups and state of residence. Women who had a previous history of ovarian cancer or a bilateral oophorectomy were ineligible controls. Among those who could be contacted, 66.5% of potential cases and 72% of potential controls agreed to participate in the main telephone interview. The study was approved by the Institutional Review Boards at all study sites.

Data were collected by a computer-assisted telephone interview, which included detailed questions on demographic factors, personal and family history of cancer, reproductive history, medication use, lifestyle characteristics, height and weight, and other factors of relevance to African-American women, such as skin pigmentation, perceived discrimination, and cultural beliefs.

Dietary assessment. We assessed individual exposure levels of antioxidant nutrients from both food sources and dietary supplements, specifically vitamins E and C, carotenoids, and selenium. Dietary information was assessed via a self-administered Block 2005 FFQ, which included questions on the usual frequency and portion size for 110 food and beverages consumed over the year preceding diagnosis for cases or the interview date for controls (11). Usual intake of dietary supplements of antioxidant nutrients, including multivitamins, was also collected. Nutrient intakes from diet and supplements were derived by Block Dietary Data Systems based on the USDA Food and Nutrient Database for Dietary Studies 1.0. Validation studies of the Block FFQ have been described elsewhere (11, 12). A previous evaluation showed that FFQ reliability was high, with Pearson correlation coefficients for

micronutrients from supplements and food ranging from 0.65 to 0.88. FFQ validity was moderate to high, with attenuated Pearson correlation coefficients for micronutrients from supplements and food ranging from 0.49 to 0.76 (11). The validity correlation for selenium for this questionnaire was 0.56.

The present study included 495 cases and 711 controls who completed the main questionnaire via telephone interview by December 2014. After excluding 72 cases and 76 controls who had not completed the FFQ for dietary assessment at time of analysis, 1 case and 3 controls who reported an extreme energy intake (greater than twice the interquartile range of log energy intake), and 16 cases subsequently determined to be noneligible by pathology review, a total of 406 cases and 632 controls remained for the analysis. We compared characteristics of women completing and not completing the FFQ and found no difference with respect to age, education, region, BMI, and smoking (data not shown).

Statistical analyses. We compared the distributions of characteristics between cases and controls using chi-square tests or t tests as appropriate. We used Spearman rank correlations to compare cases' and controls' intakes of antioxidants of interest, which were selected a priori and included total and individual carotenoids, vitamin E, vitamin C, and selenium. We used unconditional logistic regressions to estimate ORs and 95% CIs of ovarian cancer by quartile of antioxidant intake; quartiles were based on the distribution of controls. We investigated dietary (food sources), supplemental, and total antioxidant intake. For selected antioxidants, supplement intake was categorized as no use, medium, and high, where the latter 2 categories represent supplement users with intakes below and above the median, respectively. Then, a summary of total dietary plus supplement was created. The median value of each category was treated as a continuous variable in tests for linear trends. An α of 0.05 was used to determine statistical significance. All statistical analyses were 2-sided and were performed by using SAS version 9.4.

The full multivariable model was adjusted for age, geographic region (Midwest, South Central, or South Mid-Atlantic), education (high school or less, some post–high school training, college, or graduate degree), parity (0, 1–2, or >2), oral contraceptive use (never, <60, or ≥60 mo), menopause status (pre- or postmenopause), tubal ligation (no or yes), first-degree family history of breast or ovarian cancer (no or yes), BMI (in kg/m^2) calculated from self-reported weight and height 1 y before and treated as categorical variable [underweight or normal (<25), overweight (25–29.9), or obese (≥30)], smoking status (never, former, or current), recreational physical activity (0, <150, or ≥150 min/wk 1 y before), and total energy intake. Other covariates as listed in **Table 1** (e.g., alcohol consumption and hysterectomy) were evaluated but were not included in the final multivariate model because none changed the effect estimates by >10%.

We examined the association between antioxidants and ovarian cancer across strata of smoking (never, former, or current). Tests of statistical interaction were conducted by using Wald hypotheses tests about the model regression coefficients pertaining to the interaction, resulting in a Wald statistic with asymptotic chi-square distribution. The P values for the tests, with correct specification, can be read from SAS proc logistic Type 3 Analysis of Effects table and by specifying a "contrast" statement in proc logistic. We also conducted analyses between serous and nonserous cases and tested for heterogeneity by statistically comparing parameter estimates between corresponding relative risk ratios in a multinomial regression model.

Results

As expected, established ovarian cancer risk factors differed between cases and controls (Table 1). Cases were older and less likely to have children, to use oral contraceptives, to have a family history of breast or ovarian cancer, to consume alcohol, or to have had a tubal ligation. Cases and controls had similar intakes of total energy, protein, fat, and carbohydrates.

Median values of antioxidant intake were similar between cases and controls (data not shown). Among the antioxidants evaluated in this study, we only observed a statistically

TABLE 1   Demographic characteristics of African-American women with and without invasive epithelial ovarian cancer, 2010–2014[1]

	Cases (n = 386)	Controls (n = 622)	P[2]
Age, y			0.02
≤49	83 (21.5)	174 (28.0)	
50–59	142 (36.8)	235 (37.8)	
≥60	161 (41.7)	213 (34.2)	
Region			<0.001
Midwest	55 (14.2)	149 (24.0)	
South Central	111 (28.8)	144 (23.2)	
South Mid-Atlantic	220 (57.0)	329 (52.9)	
Education			0.03
≤High school	175 (45.3)	230 (37.0)	
Some post–high school training	95 (24.6)	179 (28.8)	
College or graduate degree	116 (30.1)	213 (34.2)	
Parity			0.02
0	79 (20.5)	83 (13.3)	
1–2	162 (42.0)	279 (44.9)	
3	145 (37.6)	260 (41.8)	
Oral contraceptive use			0.004
<60 mo	158 (40.9)	279 (44.9)	
≥60 mo	117 (30.3)	220 (35.4)	
Never	111 (28.8)	123 (19.8)	
Menopausal status			0.2
Premenopausal	105 (27.2)	191 (30.7)	
Postmenopausal	281 (72.8)	431 (69.3)	
Tubal ligation			0.09
No	250 (64.8)	370 (59.5)	
Yes	136 (35.2)	252 (40.5)	
Family history of breast or ovarian cancer (first-degree relative)			0.003
No	285 (73.8)	508 (81.7)	
Yes	101 (26.2)	114 (18.3)	
BMI, kg/m^2			0.3
<25 (underweight and normal)	56 (14.5)	115 (18.5)	
25–29.9 (overweight)	99 (25.6)	154 (24.8)	
≥30 (obese)	231 (59.8)	353 (56.8)	
Smoking			0.2
Never smoker	218 (56.5)	357 (57.4)	
Former smoker	101 (26.2)	138 (22.2)	
Current smoker	67 (17.4)	127 (20.4)	
Physical activity			0.07
Sedentary	139 (36.0)	200 (32.2)	
Only mild	79 (20.5)	157 (25.2)	
Some moderate	139 (36.0)	199 (32.0)	
Any strenuous	29 (7.5)	66 (10.6)	
Supplement use[3]			
Selenium	50.5	56.8	0.05
Vitamin C	60.9	65.3	0.2
Vitamin E	59.6	64.6	0.1
Total intake[4]			
Energy, kcal/d	1771 ± 1230[5]	1750 ± 1132	0.7
Ethanol, g/d	3.4 ± 18	6.1 ± 18	0.02
Carbohydrate, g/d	218 ± 158	209 ± 137	0.3
Fat, g/d	73 ± 50	73 ± 50	1.0
Protein, g/d	65 ± 47	66 ± 46	0.8

[1] Values are n (%) unless otherwise indicated. Only complete cases are shown. Missing data on education for 1 control, menopause for 2 controls, tubal ligation for 2 cases and 1 control, BMI for 2 cases and 1 control, and smoking for 17 cases and 5 controls.

[2] P values are for chi-square tests unless otherwise noted.

[3] Values are the percentage of subjects who used supplements.

[4] P values are for t tests.

[5] Mean ± SD (all such values).

significant difference in median intake between cases and controls for selenium from dietary supplements (2.86 µg/d for cases compared with 5.71 µg/d for controls; P = 0.031). In multivariable models, women who reported the highest intakes of total selenium had an ~30% lower risk of ovarian cancer than those with the lowest intakes (**Table 2**). Similar associations were observed for both dietary and supplemental selenium, although statistical significance was reached only for supplemental intake. The inverse associations with selenium intake were generally stronger in smokers (**Supplemental Table 1**). In additional analyses (data not shown), we did not observe effect modification of the association between selenium supplements and ovarian cancer over strata of dietary selenium intake (P = 0.44). There was also no association of dietary or supplemental zinc or copper intake with ovarian cancer.

Weak inverse associations of ovarian cancer risk with intakes of carotenoids were not significant (P = 0.07–0.60) (**Table 3**). Ovarian cancer risk was positively associated with β-cryptoxanthine (P = 0.03) (Table 3), but was not associated with vitamin C or E intake (**Table 4**). We did not observe appreciable differences in the results when we compared serous with nonserous tumors (data not shown).

Discussion

In this population-based ovarian cancer study of African-American women, compared with women with selenium intake in the lowest quartile, those with intake in the highest quartile had an ~30% lower risk of ovarian cancer; risk estimates were statistically significant for supplemental selenium but not for dietary (P = 0.1) or total selenium (P = 0.1). Although we observed an inverse trend with increasing intake of total carotenoids, statistical significance was lacking. We observed no association with either vitamin C or vitamin E. We observed no appreciable differences in results between serous and nonserous tumors, the 2 major histological types of ovarian carcinoma.

TABLE 2 The association of dietary and supplemental intake of selenium with ovarian cancer, African American Cancer Epidemiology Study, 2010–2014[1]

Intake, µg/d	Cases	Controls	OR[2] (95% CI)	P-linear[3]
Dietary				0.1
Q1 (<47.8)	88 (21.7)	158 (25.0)	1.00 Referent	
Q2 (47.8–72.6)	122 (30.0)	158 (25.0)	1.19 (0.74, 1.91)	
Q3 (72.7–111.7)	102 (25.1)	158 (25.0)	0.98 (0.54, 1.78)	
Q4 (>111.7)	94 (23.2)	158 (25.0)	0.66 (0.31, 1.37)	
Supplemental				0.04
T1 (nonconsumer)	203 (50.0)	274 (43.4)	1.00 Referent	
T2 (0–20.0)	139 (34.2)	211 (33.4)	0.89 (0.66, 1.21)	
T3 (>20.0)	64 (15.8)	147 (23.3)	0.67 (0.46, 0.97)*	
Total				0.1
Q1 (<60.9)	110 (27.1)	158 (25.0)	1.00 Referent	
Q2 (60.9–96.4)	120 (29.6)	158 (25.0)	0.91 (0.60, 1.38)	
Q3 (96.5–137.4)	76 (18.7)	158 (25.0)	0.58 (0.35, 0.94)*	
Q4 (>137.4)	100 (24.6)	158 (25.0)	0.67 (0.39, 1.14)	

[1] Values are n (%) unless otherwise indicated. Supplement or dietary counterpart as covariate was added when appropriate. *P < 0.05, different from the reference level. Q, quartile; T, tertile.
[2] Adjusted for age, region, education, parity, oral contraceptive use, menopause, tubal ligation, family history, BMI, smoking status, total energy, and physical activity.
[3] P-linear is for a test of linear trend treating quartile and tertile medians as a continuous variable.

TABLE 3 The association of dietary and supplemental intake of carotenoids with ovarian cancer, African American Cancer Epidemiology Study, 2010–2014[1]

Intake, µg/d	Cases	Controls	OR[2] (95% CI)	P-linear[3]
α-Carotene				0.4
Q1 (<137)	103 (25.4)	158 (25.0)	1.00 Referent	
Q2 (137–272)	105 (25.9)	158 (25.0)	0.92 (0.63, 1.36)	
Q3 (273–492)	99 (24.4)	158 (25.0)	0.86 (0.58, 1.27)	
Q4 (>492)	99 (24.4)	158 (25.0)	0.82 (0.55, 1.24)	
β-Cryptoxanthine				0.03
Q1 (<59)	93 (22.9)	158 (25.0)	1.00 Referent	
Q2 (59–99)	85 (20.9)	158 (25.0)	0.90 (0.59, 1.37)	
Q3 (100–185)	103 (25.4)	158 (25.0)	1.13 (0.75, 1.72)	
Q4 (>185)	125 (30.8)	158 (25.0)	1.45 (0.93, 2.25)	
Lutein zeaxanthin				0.4
Q1 (<1672)	131 (32.3)	158 (25.0)	1.00 Referent	
Q2 (1672–2744)	80 (19.7)	158 (25.0)	0.63 (0.42, 0.93)*	
Q3 (2745–5018)	92 (22.7)	158 (25.0)	0.72 (0.49, 1.07)	
Q4 (>5018)	103 (25.4)	158 (25.0)	0.74 (0.49, 1.10)	
Lycopene				0.2
Q1 (<1537)	100 (24.6)	158 (25.0)	1.00 Referent	
Q2 (1537–2644)	113 (27.8)	158 (25.0)	1.07 (0.72, 1.59)	
Q3 (2645–4498)	104 (25.6)	158 (25.0)	0.87 (0.57, 1.33)	
Q4 (>4498)	89 (21.9)	158 (25.0)	0.77 (0.48, 1.25)	
β-Carotene				
Dietary				0.6
Q1 (<2026)	106 (26.1)	158 (25.0)	1.00 Referent	
Q2 (2026–3560)	97 (23.9)	158 (25.0)	0.84 (0.57, 1.25)	
Q3 (3561–6003)	102 (25.1)	158 (25.0)	0.95 (0.64, 1.41)	
Q4 (>6003)	101 (24.9)	158 (25.0)	0.86 (0.56, 1.31)	
Supplemental				0.2
T1 (nonconsumer)	223 (54.9)	321 (50.8)	1.00 Referent	
T2 (0–1200.0)	171 (42.1)	283 (44.8)	0.85 (0.64, 1.12)	
T3 (>1200.0)	12 (3.0)	28 (4.4)	0.59 (0.28, 1.24)	
Total				0.2
Q1 (<2384)	106 (26.1)	158 (25.0)	1.00 Referent	
Q2 (2384–4152)	107 (26.4)	158 (25.0)	0.87 (0.59, 1.28)	
Q3 (4153–7027)	98 (24.1)	158 (25.0)	0.86 (0.58, 1.29)	
Q4 (>7027)	95 (23.4)	158 (25.0)	0.74 (0.49, 1.13)	
Total carotenoids				0.07
Q1 (<6779)	112 (27.6)	158 (25.0)	1.00 Referent	
Q2 (6779–11,158)	116 (28.6)	158 (25.0)	0.89 (0.61, 1.32)	
Q3 (11,159–17,527)	78 (19.2)	158 (25.0)	0.62 (0.40, 0.95)*	
Q4 (>17,527)	100 (24.6)	158 (25.0)	0.66 (0.42, 1.04)	

[1] Values are n (%) unless otherwise indicated. Intakes of α-carotene, β-cryptoxanthine, lutein zeaxanthin, and lycopene were measured from food sources only. Supplement or dietary counterpart as covariate was added when appropriate. *P < 0.05, different from the reference level. Q, quartile; T, tertile.
[2] Adjusted for age, region, education, parity, oral contraceptive use, menopause, tubal ligation, family history, BMI, smoking status, total energy, and physical activity.
[3] P-linear is for a test of linear trend treating quartile and tertile medians as a continuous variable.

An imbalance between free radicals (reactive oxygen species) and antioxidants results in oxidative stress, which plays a role in ovarian cancer pathogenesis by causing structural alterations in DNA directly or indirectly through the formation of genotoxic lipid peroxidation byproducts that react with DNA (3). Animal and human experimental studies have shown that surface ovarian epithelial cells contain elevated concentrations of 8-oxoguanine during ovulation, which is an important mutagenic lesion in DNA (13). It has therefore been suggested that limiting oxidative stress to the ovarian epithelium could be considered a first-line defense against ovarian cancer.

TABLE 4 The association of dietary and supplemental intake of vitamin C and vitamin E with ovarian cancer, African American Cancer Epidemiology Study, 2010–2014[1]

Intake, mg/d	Cases	Controls	OR[2] (95% CI)	P-linear[3]
Vitamin C				
Dietary				0.3
Q1 (<57.0)	114 (28.1)	158 (25.0)	1.00 Referent	
Q2 (57.0–87.1)	78 (19.2)	158 (25.0)	0.67 (0.44, 1.01)	
Q3 (87.2–142.1)	100 (24.6)	158 (25.0)	0.82 (0.54, 1.26)	
Q4 (>142.1)	114 (28.1)	158 (25.0)	1.05 (0.66, 1.69)	
Supplemental				0.6
T1 (nonconsumer)	159 (39.2)	221 (35.0)	1.00 Referent	
T2 (0–142.9)	119 (29.3)	212 (33.5)	0.80 (0.58, 1.12)	
T3 (>142.9)	128 (31.5)	199 (31.5)	0.85 (0.61, 1.18)	
Total				0.7
Q1 (<86.8)	93 (22.9)	158 (25.0)	1.00 Referent	
Q2 (86.8–170.8)	109 (26.8)	158 (25.0)	1.22 (0.82, 1.81)	
Q3 (170.9–360.0)	92 (22.7)	158 (25.0)	1.02 (0.67, 1.55)	
Q4 (>360.0)	112 (27.6)	158 (25.0)	1.15 (0.77, 1.72)	
Vitamin E (α-tocopherol)				
Dietary				0.7
Q1 (<4.1)	102 (25.1)	158 (25.0)	1.00 Referent	
Q2 (4.14–6.1)	105 (25.9)	158 (25.0)	1.02 (0.66, 1.57)	
Q3 (6.2–9.1)	99 (24.4)	158 (25.0)	0.91 (0.55, 1.52)	
Q4 (>9.1)	100 (24.6)	158 (25.0)	0.90 (0.49, 1.67)	
Supplemental				0.7
T1 (nonconsumer)	164 (40.4)	226 (35.8)	1.00 Referent	
T2 (0 to <13.5)	125 (30.8)	232 (36.7)	0.72 (0.52, 0.99)*	
T3 (≥13.5)	117 (28.8)	174 (27.5)	0.86 (0.61, 1.20)	
Total				0.8
Q1 (<6.7)	104 (25.6)	158 (25.0)	1.00 Referent	
Q2 (6.7–14.8)	80 (19.7)	158 (25.0)	0.69 (0.46, 1.05)	
Q3 (14.9–25.8)	115 (28.3)	159 (25.2)	0.99 (0.68, 1.46)	
Q4 (>25.8)	107 (26.4)	157 (24.8)	0.91 (0.61, 1.37)	

[1] Values are n (%) unless otherwise indicated. Supplement or dietary counterpart as covariate was added when appropriate. *P < 0.05, different from the reference level. Q, quartile; T, tertile.

[2] Adjusted for age, region, education, parity, oral contraceptive use, menopause, tubal ligation, family history, BMI, smoking status, total energy, and physical activity.

[3] P-linear is for a test of linear trend treating quartile and tertile medians as a continuous variable.

The anticarcinogenic effects of selenium in a wide variety of animal models prompted several decades of epidemiologic studies of cancer in humans with mixed results (14). Regarding ovarian cancer specifically, results have also been mixed. Epidemiologic studies of selenium from food and supplements (15–18), serum (19, 20), hair (21), and toenails (14) have shown both null and inverse associations. We discerned no clear pattern among those studies that might reconcile the disparate findings based on methodologic or study design features.

The biological mechanisms underlying the multiple pathways through which selenium may influence cancer development remain unclear. Recently hypothesized mechanisms include a chemopreventive action of specific selenoproteins in normal cells that, conversely, take on a promoting role in existing neoplasms (22). Whether such a dual action may help explain mixed findings in the epidemiological literature is also unclear. Unlike our study, most of the previous studies that examined selenium intake in relation to ovarian cancer risk found no association with supplemental selenium intake (15, 17, 18, 23), and one found a positive association (16). Selenium from foods was examined separately in only one study (16) and showed a statistically

significant inverse association between dietary selenium intake and ovarian cancer risk. As the authors of that study noted, however, selenium concentrations in soil vary greatly by location. Therefore, nutrient composition databases may inconsistently reflect the actual intake of selenium from food.

The inverse association we observed was stronger than in previous studies of white women, which may be caused by higher levels of nutrient deficiency and/or oxidative stress in African Americans (4, 5) and lower antioxidant intakes (6, 9). These conditions may result in more African-American women with suboptimal levels or outright deficiency. In our data, the inverse association with supplemental selenium intake was not modified by dietary intake, suggesting that ameliorating deficiency is not the primary mechanism.

Regarding deficiency, the results of several well-known clinical trials of antioxidant supplements did not show reductions in the risk of any cancer (24–26), although reductions in overall cancer rates were observed in a trial conducted in a nutritionally deficient population (27). In a study in Linxian Province (China), both serum selenium (28) and serum vitamin E (α-tocopherol) (29) were inversely associated with cancer risk. The possibility that inverse associations with antioxidants tend to be stronger in (or limited to) individuals with high levels of oxidative stress is supported by the stronger inverse associations we observed in smokers. However, we cannot exclude the possibility that our results were influenced by uncontrolled (or residual) confounding from lifestyle factors or by any of the other methodological limitations noted above.

Carotenoids are lipid-soluble, yellow-orange-red pigments found in all higher plants and some animals. Proposed chemopreventive mechanisms include antioxidant, anti-inflammation, antiangionesesis, antiproliferation, apoptosis induction, immune modulation, modulation of phase I and II enzymes, induction of cell differentiation, enhancement of gap junction communication, and others (30). The association between carotenoids and ovarian cancer risk is currently unclear in epidemiologic studies. Although study results have been mixed, prospective cohort studies have largely shown no association with the major carotenoids (17, 19, 31–33) or specifically with β-carotene (34, 35). Among the case-control studies that examined specific carotenoids, results have not been consistent regarding which showed statistically significant inverse associations (2, 15, 16, 36–44). Six of the latter studies showed inverse associations with leutin and zeaxanthin (2, 32, 36, 40, 42, 43), whereas 2 studies showed no association (15, 16). Case-control studies that examined summary measures of total carotenoids (36, 38, 41, 42, 44) have tended to show statistically significant inverse associations. However, none of the prospective cohort studies showed an association with such summary measures. Although most of the cohort studies were statistically underpowered, the results of a pooled analysis of 10 cohort studies with ~2000 cases of ovarian cancer (32) also found no association. A few studies examined the association between carotenoids and ovarian cancer by tumor subtype (15, 32, 37, 38) and more often found stronger inverse associations for mucinous tumors (15, 32, 38). Finally, we observed a positive trend for β-cryptoxanthin. A similar positive association with β-cryptoxanthin was found in one previous study (15). The authors of that study noted (p. 673) that as an oxycarotenoid or xanthophylls (C-OH), it is biologically plausible that β-cryptoxanthin may act on ovarian cells differently from other hydrocarbon carotenoids (C-H).

Vitamin E is an important lipid-soluble antioxidant, and animal and human studies have shown that it may prevent the formation of lipid peroxides, which have been observed to

induce oxidant damage of DNA (18). The prospective studies of dietary and supplemental vitamin E and ovarian cancer risk have shown mostly null results (17, 32–35, 45). The results of case-control studies are more mixed with some showing null (15, 16, 23, 34, 38) and others showing inverse associations (18, 36, 37, 42, 44). One case-control study found an inverse association with supplemental vitamin E intake that was not observed with dietary vitamin E (23). Overall, the results for vitamin E supplements have been as inconsistent as those for diet (15–18, 23, 33). To date, the number of studies that considered ovarian cancer subtype is too small to discern any related patterns in findings.

The results of studies of vitamin C intake from diet or supplements have been mostly null (15–18, 32–36, 38, 41, 45, 46), but a few studies have found inverse associations with supplemental (23) or total vitamin C intake (42, 44). There also have been some reports of higher risk of ovarian cancer in women who reported taking vitamin C supplements (17, 34), although the majority of studies have shown neither a positive nor an inverse association with risk.

The reasons for the inconsistent findings with antioxidants across studies are unclear. One possibility is that this inconsistency reflects the lack of a genuine association. Alternatively, there may be a true association, but the equivocal evidence reflects methodological challenges in addressing this question. Examples of such methodological issues include the limited validity and reliability of some dietary measures, food preparation methods that are not adequately captured in the FFQ, unquantified confounding, synergy with genetic and epigenetic factors, and the fact that different populations can have different burdens of oxidative stress and, therefore, antioxidant intake may be most valuable in subgroups of the population with the highest oxidative stress (3, 47). Future research that rigorously addresses these methodological shortcomings is needed to move this field forward.

A unique strength of our study is that we could recruit a large sample of African-American women with ovarian cancer and population-based controls from various geographic regions with diverse socioeconomic and lifestyle characteristics, which increases our ability to generalize our results to the larger African-American population. Hence, our study adds to the scarce literature on the etiology of ovarian cancer in African-American women. There are also several limitations to our study. Residual confounding is possible despite the multivariable and mutually adjusted models. We also recognize the limitations of dietary recall and selection bias in case-control studies. We did not obtain circulating levels of the studied antioxidants, which may help reduce measurement error and potential bias toward the null. Regarding selection bias, the distribution of risk factors between cases and controls in our study was in the expected directions compared with other studies among African-American women (48), which increased our confidence in the validity of our findings.

In conclusion, our data suggest that higher intake of supplemental selenium may be inversely associated with risk of ovarian cancer in African-American women. Additional studies are needed to assess dietary associations with ovarian cancer in African-American women, specifically those that include antioxidants and other nutrients that may be lacking in this population.

References

1. American Cancer Society. Cancer facts and figures. [Internet]. 2016 [cited 2017 Jan 2]. Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2016.html.

2. Bertone ER, Hankinson SE, Newcomb PA, Rosner B, Willet WC, Stampfer MJ, Egan KM. A population-based case-control study of carotenoid and vitamin A intake and ovarian cancer (United States). Cancer Causes Control 2001;12:83–90.

3. Seifried HE, McDonald SS, Anderson DE, Greenwald P, Milner JA. The antioxidant conundrum in cancer. Cancer Res 2003;63:4295–8.

4. Kalinowski L, Dobrucki IT, Malinski T. Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases. Circulation 2004;109:2511–7.

5. Morris AA, Zhao L, Patel RS, Jones DP, Ahmed Y, Stoyanova N, Gibbons GH, Vaccarino V, Din-Dzietham R, Quyyumi AA. Differences in systemic oxidative stress based on race and the metabolic syndrome: the Morehouse and Emory Team up to Eliminate Health Disparities (META-Health) study. Metab Syndr Relat Disord 2012;10:252–9.

6. Watters JL, Satia JA, Kupper LL, Swenberg JA, Schroeder JC, Switzer BR. Associations of antioxidant nutrients and oxidative DNA damage in healthy African-American and White adults. Cancer Epidemiol Biomarkers Prev 2007;16:1428–36.

7. Brunst KJ, Wright RO, DiGioia K, Enlow MB, Fernandez H, Wright RJ, Kannan S. Racial/ethnic and sociodemographic factors associated with micronutrient intakes and inadequacies among pregnant women in an urban US population. Public Health Nutr 2014;17:1960–70.

8. Watters JL, Satia JA, Kupper LL. Correlates of antioxidant nutrients and oxidative DNA damage differ by race in a cross-sectional study of healthy African American and white adults. Nutr Res 2008;28:565–76.

9. Gill JK, Franke AA, Steven Morris J, Cooney RV, Wilkens LR, Le Marchand L, Goodman MT, Henderson BE, Kolonel LN. Association of selenium, tocopherols, carotenoids, retinol, and 15-isoprostane F(2t) in serum or urine with prostate cancer risk: the multiethnic cohort. Cancer Causes Control 2009;20:1161–71.

10. Schildkraut JM, Alberg AJ, Bandera EV, Barnholtz-Sloan J, Bondy M, Cote ML, Funkhouser E, Peters E, Schwartz AG, Terry P, et al. A multicenter population-based case-control study of ovarian cancer in African-American women: the African American Cancer Epidemiology Study (AACES). BMC Cancer 2014;14:688.

11. Boucher B, Cotterchio M, Kreiger N, Nadalin V, Block T, Block G. Validity and reliability of the Block98 food-frequency questionnaire in a sample of Canadian women. Public Health Nutr 2006;9:84–93.

12. Mares-Perlman JA, Klein B, Klein R, Ritter LL, Fisher MR, Freudenheim JL. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. J Nutr 1993;123:489–501.

13. Murdoch WJ, Martinchick JF. Oxidative damage to DNA of ovarian surface epithelial cells affected by ovulation: carcinogenic implication and chemoprevention. Exp Biol Med (Maywood) 2004;229:546–52.

14. Garland M, Morris JS, Stampfer MJ, Colditz GA, Spate VL, Baskett CK, Rosner B, Speizer FE, Willett WC, Hunter DJ. Prospective study of toenail selenium levels and cancer among women. J Natl Cancer Inst 1995;87:497–505.

15. Tung KH, Wilkens LR, Wu AH, McDuffie K, Hankin JH, Nomura AM, Kolonel LN, Goodman MT. Association of dietary vitamin A, carotenoids, and other antioxidants with the risk of ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005;14:669–76.

16. Gifkins D, Olson SH, Paddock L, King M, Demissie K, Lu SE, Kong AN, Rodriguez-Rodriguez L, Bandera EV. Total and individual antioxidant intake and risk of epithelial ovarian cancer. BMC Cancer 2012;12:211.

17. Thomson CA, Neuhouser ML, Shikany JM, Caan BJ, Monk BJ, Mossavar-Rahmani Y, Sarto G, Parker LM, Modugno F, Anderson GL. The role of antioxidants and vitamin A in ovarian cancer: results from the Women's Health Initiative. Nutr Cancer 2008;60:710–9.

18. Pan SY, Ugnat AM, Mao Y, Wen SW, Johnson KC; Canadian Cancer Registries Epidemiology Research Group. A case-control study of diet and the risk of ovarian cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1521–7.

19. Helzlsouer KJ, Alberg AJ, Norkus EP, Morris JS, Hoffman SC, Comstock GW. Prospective study of serum micronutrients and ovarian cancer. J Natl Cancer Inst 1996;88:32–7.

20. Knekt P, Aromaa A, Alfthan G, Maatela J, Hakama M, Hakulinen T, Teppo L. Re: Prospective study of serum micronutrients and ovarian cancer. J Natl Cancer Inst 1996;88:1408.

21. Wadhwa SK, Kazi TG, Afridi HI, Talpur FN, Naeemullah. Interaction between carcinogenic and anti-carcinogenic trace elements in the scalp hair samples of different types of Pakistani female cancer patients. Clin Chim Acta 2015;439:178–84.

22. Hatfield DL, Yoo MH, Carlson BA, Gladyshev VN. Selenoproteins that function in cancer prevention and promotion. Biochim Biophys Acta 2009;1790:1541–5.

23. Fleischauer AT, Olson SH, Mignone L, Simonsen N, Caputo TA, Harlap S. Dietary antioxidants, supplements, and risk of epithelial ovarian cancer. Nutr Cancer 2001;40:92–8.

24. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 1994;330:1029–35.

25. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145–9.

26. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, WIlliams JH, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.

27. Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 1993;85:1483–92.

28. Mark SD, Qiao YL, Dawsey SM, Wu YP, Katki H, Gunter EW, Fraumeni JF, Blot WJ, Dong ZW, Taylor PR. Prospective study of serum selenium levels and incident esophageal and gastric cancers. J Natl Cancer Inst 2000;92:1753–63.

29. Taylor PR, Qiao YL, Abnet CC, Dawsey SM, Yang CS, Gunter EW, Wang W, Blot WJ, Dong ZW, Mark SD. Prospective study of serum vitamin E levels and esophageal and gastric cancers. J Natl Cancer Inst 2003;95:1414–6.

30. Tanaka T, Shnimizu M, Moriwaki H. Cancer chemoprevention by carotenoids. Molecules 2012;17:3202–42.

31. Fairfield KM, Hankinson SE, Rosner BA, Hunter DJ, Colditz GA, Willett WC. Risk of ovarian carcinoma and consumption of vitamins A, C, and E and specific carotenoids: a prospective analysis. Cancer 2001;92:2318–26.

32. Koushik A, Hunter DJ, Spiegelman D, Anderson KE, Buring JE, Freudenheim JL, Goldbohm RA, Hankinson SE, Larsson SC, Leitzmann M, et al. Intake of the major carotenoids and the risk of epithelial ovarian cancer in a pooled analysis of 10 cohort studies. Int J Cancer 2006;119:2148–54.

33. Silvera SA, Jain M, Howe GR, Miller AB, Rohan TE. Carotenoid, vitamin A, vitamin C, and vitamin E intake and risk of ovarian cancer: a prospective cohort study. Cancer Epidemiol Biomarkers Prev 2006;15: 395–7.

34. Crane TE, Khulpateea BR, Alberts DS, Basen-Engquist K, Thomson CA. Dietary intake and ovarian cancer risk: a systematic review. Cancer Epidemiol Biomarkers Prev 2014;23:255–73.

35. Kushi LH, Mink PJ, Folsom AR, Anderson KE, Zheng W, Lazovich D, Sellers TA. Prospective study of diet and ovarian cancer. Am J Epidemiol 1999;149:21–31.

36. Bidoli E, La Vecchia C, Talamini R, Negri E, Parpinel M, Conti E, Montella M, Carbone MA, Franceschi S. Micronutrients and ovarian cancer: a case-control study in Italy. Ann Oncol 2001;12:1589–93.

37. Chiaffarino F, Parazzini F, Bosetti C, Franceschi S, Talamini R, Canzonieri V, Montella M, Ramazzotti V, Franceschi S, La Vecchia C. Risk factors for ovarian cancer histotypes. Eur J Cancer 2007;43: 1208–13.

38. Cramer DW, Kuper H, Harlow BL, Titus-Ernstoff L. Carotenoids, antioxidants and ovarian cancer risk in pre- and postmenopausal women. Int J Cancer 2001;94:128–34.

39. Huncharek M, Klassen H, Kupelnick B. Dietary beta-carotene intake and the risk of epithelial ovarian cancer: a meta-analysis of 3,782 subjects from five observational studies. In Vivo 2001;15:339–43.

40. Jeong NH, Song ES, Lee JM, Lee KB, Kim MK, Cheon JE, Lee JK, Son SK, Lee JP, Kim JH, et al. Plasma carotenoids, retinol and tocopherol levels and the risk of ovarian cancer. Acta Obstet Gynecol Scand 2009;88:457–62.

41. McCann SE, Freudenheim JL, Marshall JR, Graham S. Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. J Nutr 2003;133:1937–42.

42. McCann SE, Moysich KB, Mettlin C. Intakes of selected nutrients and food groups and risk of ovarian cancer. Nutr Cancer 2001;39:19–28.

43. Zhang M, Holman CD, Binns CW. Intake of specific carotenoids and the risk of epithelial ovarian cancer. Br J Nutr 2007;98:187–93.

44. Zhang M, Lee AH, Binns CW. Reproductive and dietary risk factors for epithelial ovarian cancer in China. Gynecol Oncol 2004;92:320–6.

45. Chang ET, Lee VS, Canchola AJ, Clarke CA, Purdie DM, Reynolds P, Anton-Culver H, Bwerstein L, Deapen D, Peel D, et al. Diet and risk of ovarian cancer in the California teachers study cohort. Am J Epidemiol 2007;165:802–13.

46. Tzonou A, Hsieh CC, Polychronopoulou A, Kaprinis G, Toupadaki N, Trichopoulou A, Karakatsani A, Trichopoulos D. Diet and ovarian cancer: a case-control study in Greece. Int J Cancer 1993;55:411–4.

47. Collins AR. Antioxidant intervention as a route to cancer prevention. Eur J Cancer 2005;41:1923–30.

48. Moorman PG, Palmieri RT, Akushevich L, Berchuck A, Schildkraut JM. Ovarian cancer risk factors in African-American and white women. Am J Epidemiol 2009;170:598–606.